天然抗氧化劑之作用機轉

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一、前言

細胞膜脂質過氧化(Lipid Peroxidation)被證實是造成人類老化、心血管疾患、癌症...等等大部份現代文明病的主要致病因子，而自由基(Free Radical)是造成脂質過氧化的毒性物質。自由基爲具有不配對電子或含奇數電子的物質的總稱，親和力極強，會攻擊不飽和脂肪酸(Polyunsaturated Fatty Acid)，造成脂質過氧化、蛋白變性、細胞變異等等。人體內有許多酵素可清除特定的自由基，例如Superoxide Dismutase (SOD)可將氧陰離子($\cdot O_2^-$)代謝成過氧化氫(H_2O_2)，而Catalase和Glutathione可將H_2O_2代謝成H_2O，但從體外攝入的化學性及藥物性物質經代謝後所產生的毒性自由基，例如Acetaminophen(普拿疼)被氧化代謝成 N－acetyl－p－benzoquinoneimine、Isoniazid(抗結核藥)被氧化代謝成Acetyl radical等等，則無相對的藥物可將之清除，但天然界存在著許多抗氧化劑，可清除代謝超氧自由基(Peroxy Radical)，例如 Vitamin C、Tocopherols、Tocotrienols、Carotenoids、Coenzyme Q、Polyphenol compounds (Flavonoids、Tannins、Cyanidins、Procyanidins)、Cytokinins等。

a　What is a free radical ?

- A reactive atom, molecule or ion possess *one unpaired electron*
- Any chemical species contains an *odd number of electrons*

Atom		Configuration	# Electron	Molecule	Free Radical
Hydrogen	H	$(1S)^1$	1	–	Yes
			2	H_2	No
Nitrogen	N	$(1S)^2(2S)^2(2P)^3$	7	–	Yes
Oxygen	O	$(1S)^2(2S)^2(2P)^4$	8	–	Triplet
			9	$\cdot OH$	Yes
			14	N_2	No
			15	$\cdot NO$	Yes
			16	O_2	No
			17	$\cdot O_2^-$	Yes

a　Formation Pathways of Free Radicals in Biological Systems

(A) Biosynthesis

→ Production of *nitric oxide* (NO) from L-Arginine via NO-synthase

$\cdot N{=}O$　　$\cdot N{=}O + \cdot O_2^- \rightarrow O{=}N{-}O{-}O{-}$

NO is a potent biological effector, and by definition, is also a free radical

Peroxynitrite, a powerful oxidant and nitrating agent

(B) Metabolism

※ **Formation of superoxide anion radicals ($\cdot O_2^-$)**

1) Spontaneous autoxidation of electron transport carriers in mitochondria and endoplasmic reticulum
2) Oxidation of hemoglobin
3) Activation of phagocyte
4) Xanthine／xanthine oxidase

※ **Formation of hydroxyl radical ($\cdot OH$)**

1) Fenton reaction　　2) Haber-Weiss reaction

$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$ ($\cdot O_2^-$ reducing the iron salt)

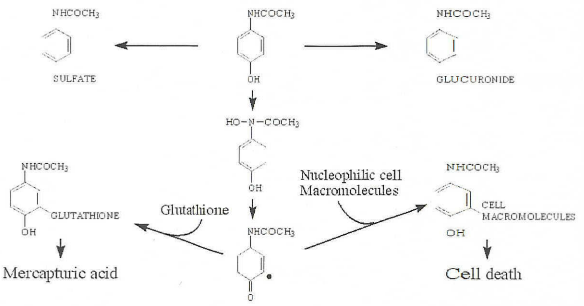
$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$

Net：$\cdot O_2^- + H_2O_2 \rightarrow O_2 + OH^- + \cdot OH$

(An iron-catalyst Haber-Weiss reaction)

(C) Environmental effects

1) Air pollution
2) High energy radiation → UV, x-ray, γ-ray, etc.
3) Foods and drugs contamination



二、Vitamin C

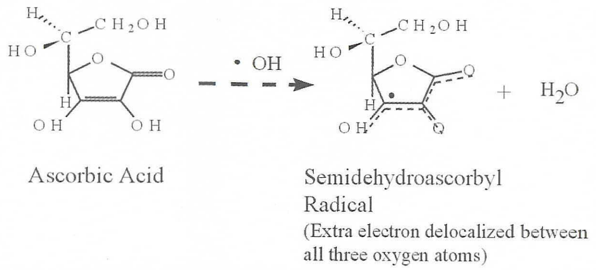
維生素C屬水溶性維生素，在構成內皮細胞間之膠原纖維(Collagen Fiber)合成方面，維生素C在Proline和Lysine氫氧化(Hydroxylation)過程中是爲還原劑。

L－Ascorbic acid爲活性型，D－Ascorbic acid不具生理活性。維生素C可被氧化形成Dehydroascorbic acid (Oxidized form)，此反應爲可逆，但Dehydroascorbic acid可

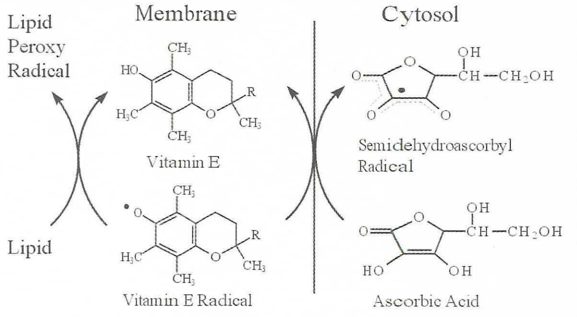
被水解成2,3—diketo—L—gulonic acid，此反應不可逆，且在中性及鹼性溶液中，此水解反應自然產生，維生素C經此水解就失去活性，因此，保存溶液在酸性則可保存維生素C的活性，此外，乾燥後之蔬菜會失去其所含之維生素 C，而維生素C易被氧化，特別在有銅金屬存在時，因此煮器勿用銅器。

維生素 C 可以清除・ＯＨ 自由基，在反應中，維生素 C 被氧化成毒性低且水可溶的Semidehydroascorbyl Radical而排出體外。此外，在細胞膜上之維生素E被氧化成VitaminE Radical時，在細胞漿質內，維生素C可將氧化型的Vitamin E Radical還原成維生素E，而維生素C本身被氧化成Semidehydroascorbyl Radical。

The Mechanism of Scarvenging Hydroxy Radical (・OH) with Ascorbic Acid



Interaction Between Vitamin C and Vitamin E



三、Vitamin E (—Tocopherol)

Tocopherol為脂溶性維生素，有α—, β—, γ—, δ—form，Vitamin E指的為α—Tocopherol，是生理活性最強的Tocopherol。維生素E的生物活性單位有IU和mg，轉換如下：

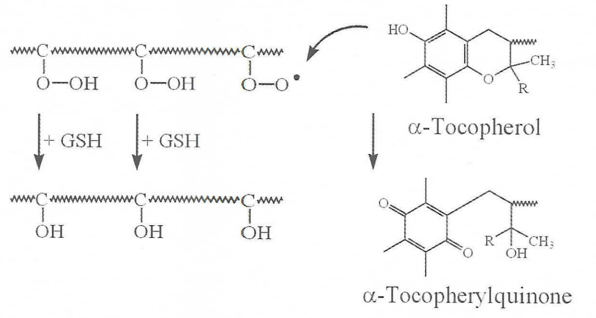
a) 1 mg d—α—Tocopherol (Naturally occurring form) ＝ 1.49 IU

b) 1 mg d—α—Tocopheryl acetate (Natural) ＝ 1.36 IU

c) 1 mg dl—α—Tocopherol (Synthetic) ＝ 1.1 IU

維生素E是舉世公認的抗氧化劑，許多抗氧化藥物的活性研究都以維生素E為對照藥物。維生素E的抗氧化活性和清除自由基有關，不論是・OH、・O^{2-}、H_2O_2或脂肪之過氧化物，維生素E都能清除之。

Role of Vitamin E in the Cellular Defense Against Lipid Peroxidation



a Comparison of Some Representative Foods as Sources of Vitamin E. (*mg/100 g of Food*)

Food	*Total Vitamin E*	*Alpha Tocopherol*	*Total Active Tocopherols**
Oils			
coconut	3.58	0.35	0.78
cod liver, commercial	21.96	21.96	21.96
corn, refined	83.17	14.26	21.10
cottonseed , refined	65.24	35.26	38.26
olive	12.64	11.92	11.99
palm , refined	35.53	18.32	21.82
peanut , refined	25.00	11.62	12.92
rapeseed , refined	44.81	17.65	20.35
safflower seed , refined	38.10	34.05	34.40

* Expressed as α-tocopherol.

a Comparison of Some Representative Foods as Sources of Vitamin E. (*mg/100 g of Food*)-------- *Continued (1)*

Food	*Total Vitamin E*	*Alpha Tocopherol*	*Total Active Tocopherols**
Oils----continued			
sesame seed , refined	29.07	1.38	4.07
soybean, refined	93.74	10.99	17.43
sunflower seed , refined	63.62	59.50	59.85
wheat germ	254.58	149.44	183.00
wrasse liver	250.67	250.67	250.67
Other foods			
peanut, raw	16.37	8.33	9.13
pecan, raw	19.86	1.24	3.10

* Expressed as α-tocopherol.

a Comparison of Some Representative Foods as Sources of Vitamin E. (*mg/100 g of Food*) -------- *Continued (2)*

Food	*Total Vitamin E*	*Alpha Tocopherol*	*Total Active Tocopherols**
Other foods----continued			
spinach, raw	3.00	1.88	1.90
tomato, raw	0.49	0.34	0.35
walnut, English, raw	19.62	0.84	2.63
wheat germ	27.56	14.07	17.38
milk			
cow, commercial	0.09	0.06	0.06
human	0.99	0.88	0.90
margarine, soybean and cottonseed oils -- stick	45.49	11.15	13.87

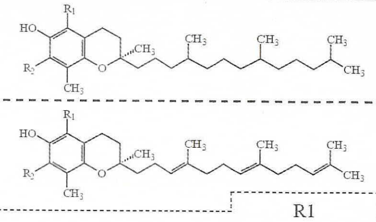
* Expressed as α-tocopherol.

a Comparison of Some Representative Foods as Sources of Vitamin E. (*mg/100 g of Food*) -------- *Continued (3)*

Food	*Total Vitamin E*	*Alpha Tocopherol*	*Total Active Tocopherols**
Other foods----continued			
almond, raw	24.48	23.96	24.01
bean			
kidney, dry	2.08	tr	0.21
soy, dry	20.43	0.85	2.03
beef, skeletal muscle, raw	0.43	0.41	0.41
bread, white, U.S.	1.19	0.12	0.20
butter, U.S.	1.58	1.58	1.58
carrot, raw	0.51	0.44	0.46
eggs, whole large, raw	1.06	0.70	0.74

* Expressed as α-tocopherol.

Structures of Various Homologs of Tocopherol and Tocotrienol



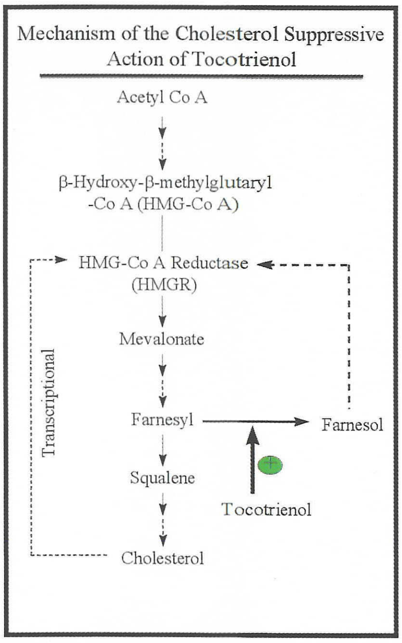
Tocopherol

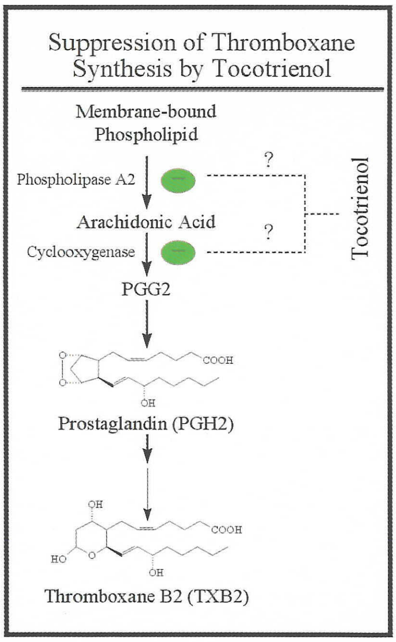
Tocotrienol

	R1	R2
α -Tocopherol / Tocotrienol	CH_3	CH_3
β -Tocopherol / Tocotrienol	CH_3	H
γ -Tocopherol / Tocotrienol	H	CH_3
δ -Tocopherol / Tocotrienol	H	H

四、Tocotrienol

馬來西亞棕櫚油研究中心（Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia)從棕櫚油(Palm oil；PalmVitee)中分餾得到富含Vitamin的物質，具有極強的抗氧化活性，這些物質爲維生素E家族的成員，稱爲Tocopherols和Tocotrienols，結構上Tocopherols和Tocotrienols具有相同的Chromanol head，在C-2位置也都具有相同碳數和構造的支鏈，唯Tocopherol的支鏈爲飽和的Phytyl tail，而Tocotrienol爲具有三個雙鍵的不飽和Isoprenoid side chain。

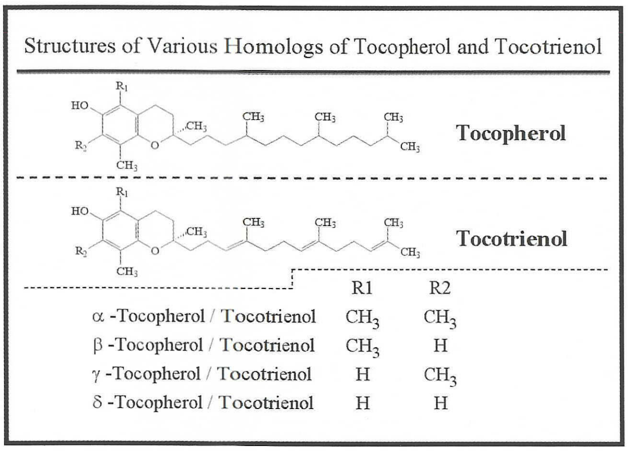


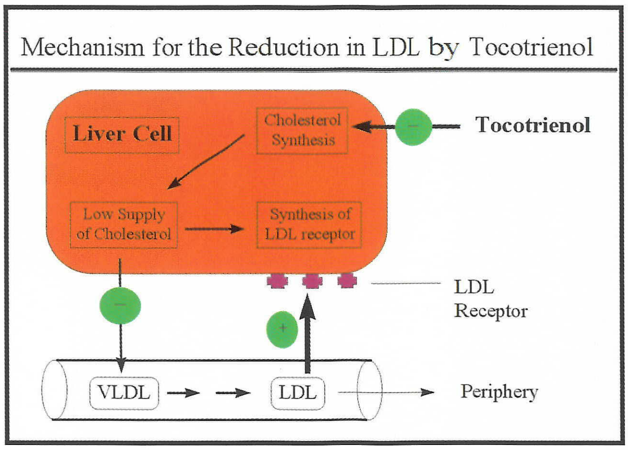


Comparison of Change in Carotid Stenosis

	Antioxidant	Placebo
6 months [a]		
MR	1	0
R	5	0
NC	18	20
P	1	2
MP	0	3
Total Number	25	25
12 months [a]		
MR	1	0
R	6	0
NC	16	15
P	2	6
MP	0	4
Total Number	25	25

[a] Data expressed as number of subjects per category.
MR, marked regression; R, regression;
NC, no change; P, progression;
MP, marked progression.





Tocotrienols可在穀物(例如大麥、燕麥、黑麥)和蔬菜油(例如棕櫚油、米糠油、橄欖油)取得，抗氧化活性比Tocopherol還強。藥理研究證實 Tocotrienols可降低膽固醇、防止動脈血管壁增厚，其機轉爲防止低密度膽固醇(LDL)被自由基氧化，以避免氧化型低密度膽固醇(Oxidized-LDL)沉積在動脈血管內皮組織而使血管內壁增厚，此外，Tocotrienols可抑制肝臟內HMG-Co A reductase之活性，而直接抑制膽固醇的合成，由於Tocopherol (Vitamin E)並不具有降低膽固醇的作用，因此Tocotrienols在心血管疾患的預防及治療，近年來頗受到世人的重視。

Tocotrienols在抗腫瘤方面也有很大的癌細胞毒殺活性，研究上指出Tocotrienols可抑制下列腫瘤細胞株的增生(Proliferation)：B16 melanoma cells， MDA-MB-231 和 MDA-MB-435 breast cancer cells，H69 lung carcinoma，HeLa cervical epitheloid carcinoma， P388 leukemia cells等，而γ-Tocotrienol對植入大鼠體內的B16 melanomas更有直接抑制生長的作用，其機轉爲誘導癌細胞之自然凋亡(Apoptosis) 和停止細胞週期(Cell-Cycle Arrest)。

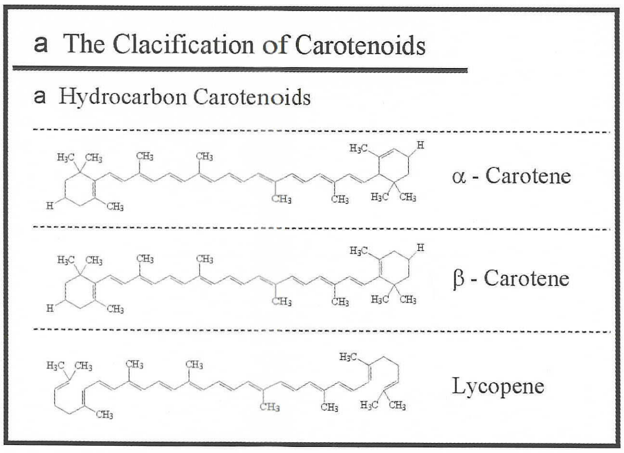
結構探討方面，不具甲基的 d – 2-Desmethyl tocotrienol具最強的癌細胞毒殺活性，而δ-Tocotrienol 不論在毒殺腫瘤細胞和抗氧化活性方面，其活性均大於α – 和 γ – form 之Tocotrienols。

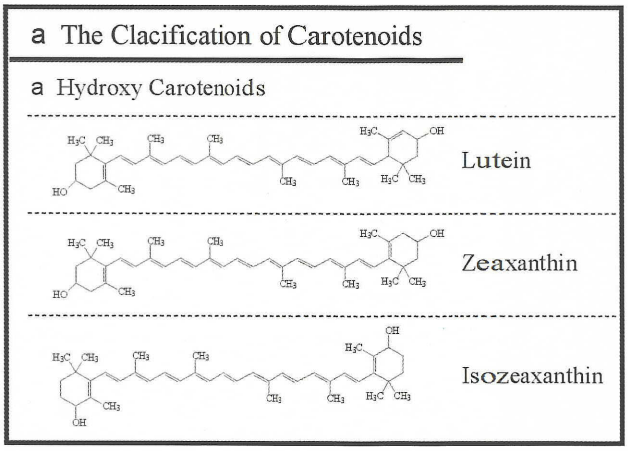
五、Carotenoids

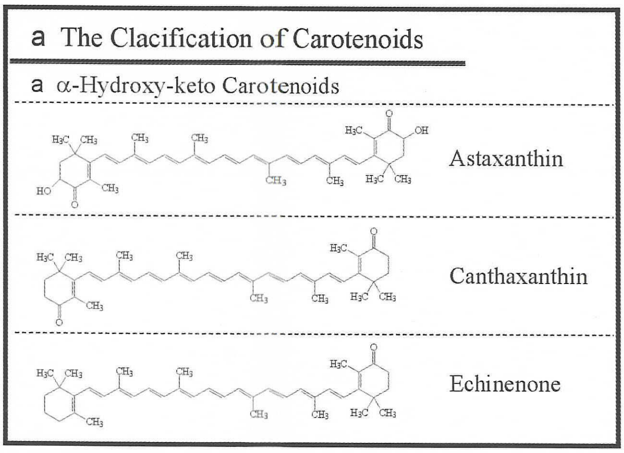
類胡蘿蔔素(Carotenoids)爲一種脂溶性的抗氧化劑，存在於綠色、黃色蔬菜及黃色果實中，在人體小腸經膽酸(Bile acid)的協助而被吸收，之後在肝臟代謝轉變成維生素A而供人體利用，因此，Carotenoids又被稱爲原維生素A (Provitamin A)。

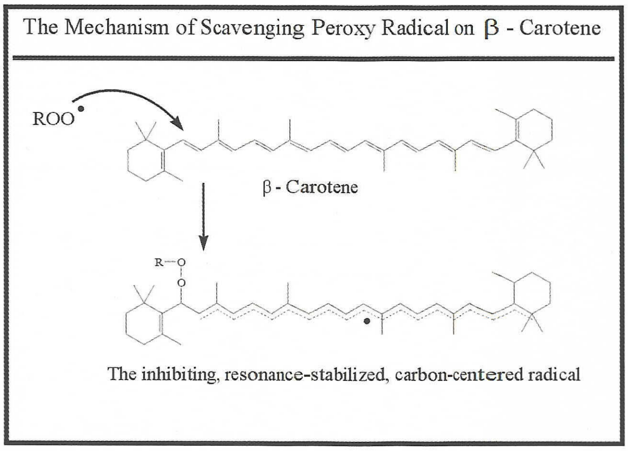
研究上常用的Carotenoids分類如下：(1)Hydrocarbon Carotenoids，如α－、β－Carotene、Lycopene； (2)Hydroxy 主要 Carotenoids，如 Lutein、 Zeaxanthin、I sozeaxanthin 等；(3)－Hydroxy －ketocarotenoids，如 Astaxanthin、Canthaxanthin、Echinenone 等。人類 血清中的Carotenoids爲α－Carotene、β－Carotene、Lycopene、Lutein、Zeaxanthin、Cryptoxanthin等，但在視網膜上僅有Lutein和Zeaxanthin可被偵測到，此乃因這二種Carotenoids能穿越視網膜的血壁障礙而清除$^1O_2$自由基，具有保護視網膜的作用。

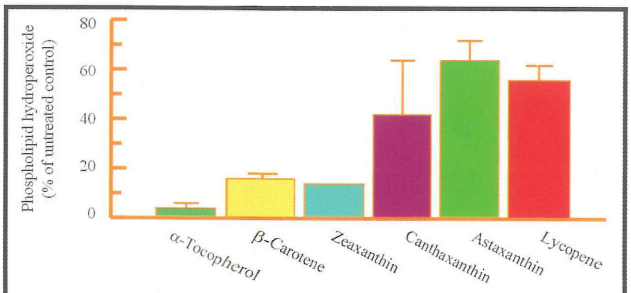
Carotenoids可以消除Singlet Oxygen($^1O_2$)和自由基(Free Radical)，在動脈血管粥狀硬化、老化、癌症、眼疾患等，Carotenoids 具有延緩 (Delaying)和預防(Preventing)的作用 。在這些Carotenoids中，抗氧化能力大約爲Zeaxanthin、β－Cryptoxanthin＞β－Carotene＞Astaxanthin、Canthaxanthin，其中Zeaxanthin之活性甚至和Vitamin E不相上下。對Peroxy Radicals(ROO‧或R‧)而言，Carotenoids會捕捉這些自由基，與之結合形成以碳原子爲中心之穩定共震自由基複合物，而達成清除自由基之目的。



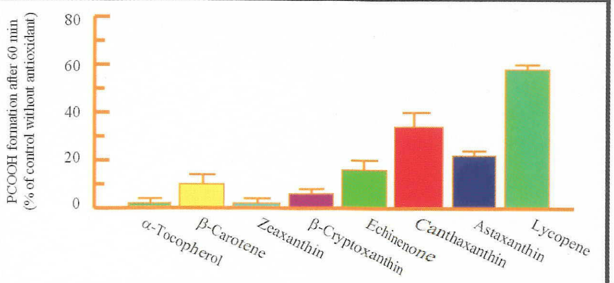








Comparison of the effects of a-tocopherol and carotenoids (1 mol%) on the formation of phospholipid hydroperoxides by reaction of EYPC (5 mM) with peroxyl radicals generated via AMVN in homogenous organic solution, in air at 37℃.



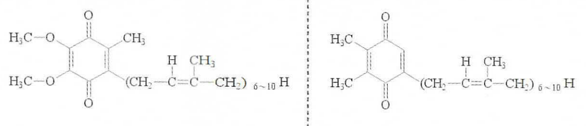
Comparison of the effects of a-tocopherol and carotenoids (1 mol%) on the formation of phospholipid hydroperoxides by reaction of EYPC (5 mM) with peroxyl radicals generated via AMVN in liposomes organic solution, in air at 50℃.

六、Coenzyme Q

哺乳類動物的呼吸器官會合成一種輔酵素 Q(Coenzyme Q)，被稱爲Ubiquinone，簡寫爲Co Q，基本骨架屬於Benzoquinone，具有四個取代基，其中一個取代基爲由6～10個Isoprenoids組成之疏水性長鏈，因此，Coenzyme Q也屬於脂溶性的輔酵素。在粒線體的膜上，Coenzyme Q擔任電子轉移的功能，而在植物葉綠體則存在著一種Ubiquinone的類似物，稱爲Plastoquinone，也具有相同的電子轉移的功能。

Coenzyme Q爲強力氧化劑，可被NADH或FADH2還原。在氧化－還原反應中，Coenzyme Q能接受或供給一個或兩個電子，因此在氧化－還原反應中，Coenzyme Q 呈現三種不同的型態：氧化型的Q、半還原型的自由基、還原型的QH2(又稱爲Ubiquinol；Q10)。在粒線體的電子轉移過程，半還原型的自由基呈現爲·Q^{-}的陰離子。

Q10生理上可保護細胞膜蛋白質DNA之脂質過氧化所造成的傷害，不論是氧化型的Ubiquinone或還原型的Ubiquinol，都具有抗氧化的活性，對人體而言，Coenzyme Q 可抑制 LDL 被氧化，因此Coenzyme Q被認爲可防止動脈血管粥狀硬化之產生。



Ubiquinone　　Plastoquinone

Structure of Ubiquinone and Plastoquinone. The Hydrophobic Tail of Each Molecule is Composed of Five-carbon Isoprenoid Units.

$$2 \cdot O_2^- + CoQH_2 \longrightarrow H_2O_2 + O_2 + CoQ$$

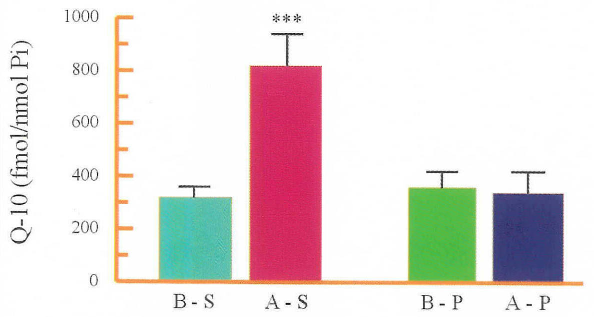
$$2\ L^{\cdot} + CoQH_2 \longrightarrow 2\,LH + CoQ$$

$$2\ LOO\cdot + CoQH_2 \longrightarrow 2\,LOOH + CoQ$$

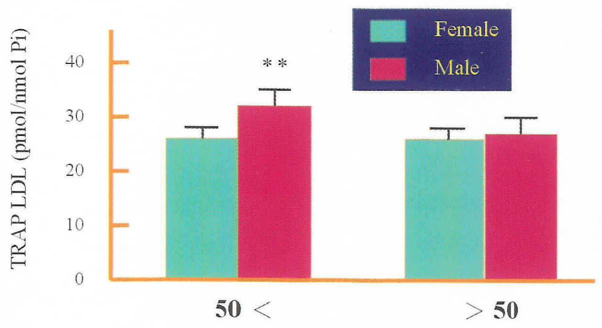
TBA-Reacting Material (MDA) in Liver Fractions from Rats Treated with CCl_4, Ethanol, and CoQ. Data as Mean ± SE.

Group	Fraction: Homog. [a]	Mito. [b]	Mic. [b]
Control	86±4	8.2±0.3	5.4±0.3
CoQ	13±1 (p<.001)	3.7±0.3 (p<.01)	3.2±0.1 (p<.02)
CCl_4	766±14 (p<.001)	28.3±2.8 (p<.05)	10.7±0.8 (p<.05)
CCl_4 + CoQ	465±13 (p<.001)	11.1±0.7 (p<.05)	6.5±0.4 (p<.1)
EtOH	188±7 (p<.001)	19.8±2.2 (p<.05)	8.4±0.4 (p<.05)
EtOH + CoQ	15±2 (p<.001)	4.6±0.4 (p<.02)	3.4±0.5 (p<.01)

[a] nmol MDA/gram liver.　[b] nmol MDA/mg protein.



The LDL-Q_{10} Concentration Before (B) and After (A) Supplementation (100 mg/day, 30 days) in the Supplemented (B-S, B-A) and Placebo (B-P, A-P) Group (P=0.00006).



The Age and Gender Differences in $TRAP_{LDL}$ (P = 0.019).

Effect of Redox State of Coenzyme Q on NADPH, ADP-Fe^{2+}-Supported Lipid Peroxidation Catalyzed by Rat Liver Microsomes

Additions	Concentration (μM)	O_2 Consumption (natoms/min/mg protein)	MDA formed (nmol)
EtOH－CL [a]	—	224 (100) [b]	5.87 (100) [b]
CoQ10	150	320 (143)	5.71 (97)
$CoQ10H_2$	150	59 (26)	1.19 (20)
$CoQ10H_2$	300	31 (14)	0.26 (4)
CoQ10 ＋ $CoQ10H_2$	150 ＋ 75	97 (43)	2.01 (34)

[a] Abbreviations：C, control; CL, cardiolipin.
[b] The number in parentheses indicates % of control.

The Content of CoQ10 in Selected Food Items and the Contribution from the Food Groups to the Average Daily Intake.

Food Group	Food item	n	Cooking	CoQ10 μg/g food	Intake g/day	CoQ10 mg/day
Meat and poultry	Pork heart	9	Fried	203	120	2.2－3.2
	Beef	1	Fried	31		
	Chicken	1	Fried	17		
	Pork chop	3	Fried	14		
	Ham	3	Boiled	7.7		
Fish	Herring	1	Marinated	27	26	0.4
	Rainbow trout	1	Steamed	11		
	Salmon	1	Smoked	4.3		

n.d., below detection limit; n, number of samples. The values represent means of triplicate measurements. The range of different samples are given in parentheses.

The Content of CoQ10 in Selected Food Items and the Contribution from the Food Groups to the Average Daily Intake. ---- *continued*

Food Group	Food item	n	Cooking	CoQ10 μg/g food	Intake g/day	CoQ10 mg/day
Fruit	Orange	1	None	2.2	154	0.2
	Apple	1	None	1.1		
	Kiwi	1	None	0.49		
Dairy products	Yogurt	1	None	1.2	426	0.1－0.5
	Hard cheese	1	None	＜0.16		
	Cream cheese	1	None	＜0.29		
Egg	Hens egg	2	None	1.5	36	0.06－0.08
	Hens egg	2	Boiled	2.3		
Dietary fat	From (Kamei et al., 1986)			[3－7]	79	0.2－0.3

n.d., below detection limit; n, number of samples. The values represent means of triplicate measurements. The range of different samples are given in parentheses.

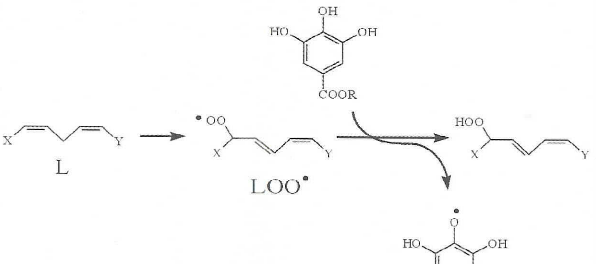
The Content of CoQ10 in Selected Food Items and the Contribution from the Food Groups to the Average Daily Intake. ---- *continued*

Food Group	Food item	n	Cooking	CoQ10 μg/g food	Intake g/day	CoQ10 mg/day
Cereals and vegetables	Bread (rye)	1	None	＜0.17	227	0－0.03
	Bread (wheat)	1	None	＜0.09		
	Rice	1	Boiled	＜0.18		
	Broccoli	3	Boiled	6.6	270	0.3
	Cauliflower	1	Boiled	4.9		
	Potato	1	Boiled	0.52		
	Tomato	1	None	0.19		
	Carrot	1	None	＜0.24		
	Carrot	1	Boiled	＜0.21		
	Cucumber	1	None	＜0.08		

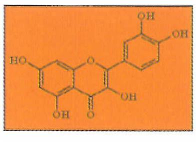
n.d., below detection limit; n, number of samples. The values represent means of triplicate measurements. The range of different samples are given in parentheses.

七、Polyphenol compounds (Flavonoids、Tannins、Cyanidins、Procyanidins)

多酚類化合物 (Polyphenol compounds) 如黃酮類、單寧、花青素、原花青素等等，具有還原性酚基，是極強的清除自由基、抗氧化的物質，在多酚類成分中所具有的羥基氫極爲活潑，具有極強的供氫能力，能提供電子給脂肪酸自由基，使脂肪酸自由基轉化爲惰性物質，終止自由基的連鎖反應，終止脂肪酸的自發性氧化。



The Mechanism of Anti-lipid Peroxidation on Tannin



I：5-hydroxy-3, 6, 7, 3□4□pentamethoxyflavone (Artemetin)
II：5, 6, 4□trihydroxy-7, 8, 3□trimethoxyflavone (Thymonin)
III：5, 4□dihydroxy-3, 6, 7-trimethoxyflavone
IV：5, 4□dihydroxy-6, 7, 8, 3□tetramethoxyflavone
V：3, 3□4□5, 7-pentahydroxyflavone (Quercetin)
VI：5, 6, 7, 8, 2□3□4□5□octamethoxyflavone (Agehoustin A)
VII：3, 3□4□5, 7-pentahydroxyflavone-3-rutinoside(Rutin)

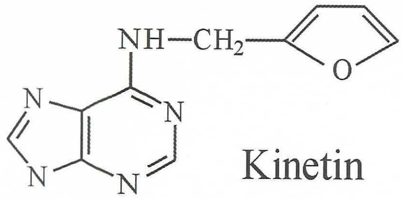
Compound	3	5	6	7	8	2□	3□	4□	5□
I	OMe	OH	OMe	OMe	H	H	OMe	OMe	H
II	H	OH	OH	OMe	OMe	H	OMe	OH	H
III	OMe	OH	OMe	OMe	H	H	H	OH	H
IV	H	OH	OMe	OMe	OMe	H	OMe	OH	H
V	H	OMe	OMe	OMe	OMe	OMe	OMe	OMe	OMe
VI	OH	OH	H	OH	H	H	OH	OH	H
VII	ORut*	OH	H	OH	H	H	OH	OH	H

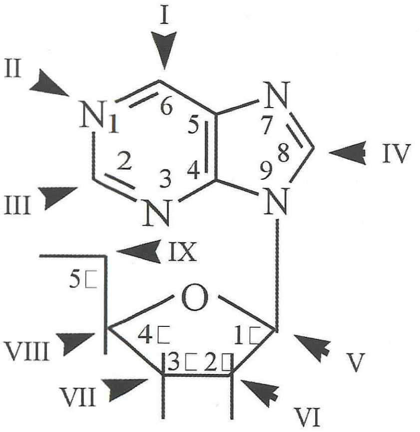
* Rut：rutinose (＝Glu－Rha).

Relative and Comparative TOSC Values for the Different Flavonoids Tested.

	rTOSC	cTOSC
Trolox	5.62	1
I：5-hydroxy-3, 6, 7, 3□4□pentamethoxyflavone (Artemetin)	1.55	0.28
II：5, 6, 4□trihydroxy-7, 8, 3□trimethoxyflavone (Thymonin)	8.53	1.52
III：5, 4□dihydroxy-3, 6, 7-trimethoxyflavone	1.21	0.22
IV：5, 4□dihydroxy-6, 7, 8, 3□tetramethoxyflavone	4.53	0.81
V：3, 3□4□5, 7-pentahydroxyflavone (Quercetin)	40.32	7.17
VI：5, 6, 7, 8, 2□3□4□5□octamethoxyflavone (Agehoustin A)	0.13	0.02
VII：3, 3□4□5, 7-pentahydroxyflavone-3-rutinoside (Rutin)	28.96	5.15

八、Kinetin


Kinetin



The possible sites of hydroxyl radicals attack on purine nucleosides in DNA and damage products which can be formed in these reactions.

I：2′-deoxyinosine,

II：N1 oxide of adenosine (non radical product),

III：2-hydroxyadenosine,

IV：8-hydroxydeoxyguanosine or 8-hydroxy-deoxyadenosine,

V：hydroxylation at C1′ leads to 5-methylene-2-furanone,

VI：formation of ribonucleotides (2′ hydroxylation; 2′ oxidation leads to the release of the base),

VII：abstraction of hydrogen at the carbon 3′ (the C3′ chemistry might be involved in DNA cleavage),

VIII：hydroxylation at the carbon C4′, after cleavage it gives 5′ phosphorylated terminus and a 3′ terminus bearing the sugar residue,

IX：abstraction of hydrogen at C5′ leads to the formation of furfural.

Cellular and Biochemical Characteristics of Ageing Human Skin Fibroblast Cultures Maintained in the Presence or Absence of Kinetin

Characteristic	Untreated cultures		Kn-treated cultures	
	early passage	late passage	early passage	late passage
PD time (days)	8±1	20±2	8±1	15±3
Cell yield (104 cells/cm2)	4±0.5	1±0.2	4±0.5	3±0.3
DNA synthesis (104 cpm/106 cells)	35±4	5±0.5	39±5	5±0.4
S-phase positive cells (%)	92±4	5±1	93±3	8±3
RNA synthesis (103 cpm/106 cells)	5±0.4	3±0.5	5±0.5	3±0.4
Protein synthesis (106 cpm/106 cells)	4±2	1±0.6	4±2	3±2
Protein content (mg/106 cells)	325±50	985±70	320±60	535±45
Autofluorescence	low	high	low	low
Actin filaments	diffused	highly polymerized	diffused	less polymerized
Microtubules	orderly	disorganized	orderly	orderly